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Generation of hazard indices for cumulative exposure to phthalates for use in cumulative risk assessment

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ABSTRACT

Exposures to multiple chemicals may contribute to increased risk of similar adverse effects. Cumulative risk may be estimated using a hazard index (HI), the sum of individual hazard quotients (HQ, ratio of exposure to the reference value). We demonstrate the HI approach for five phthalates: di(2-ethylhexyl) phthalate (DEHP), di-n-butyl phthalate (DBP), diisobutyl phthalate (DiBP), diisononyl phthalate (DiNP), and butyl benzyl phthalate (BBP). Phthalate exposure for the US general population is estimated using urine metabolite levels from NHANES, extrapolating to ingested 'dose' using the creatinine correction approach. We used two sets of reference values: European Union Tolerable Daily Intakes and Denmark Environmental Protection Agency Derived No Effect Levels. We also investigated the use of an alternate reference value for DEHP, derived from a recent study on male reproductive system development. HQs and HIs were calculated for the total population ages 6 years and older, as well as for men and women of approximate reproductive age (18–39 years), and children (6–11 years). Median HQs ranged from <0.01 for BBP, to ~0.1 (using established values) or ~2 (using an alternate value) for DEHP. Median HIs were <0.30 (95th percentiles just >1.0), and were driven by DEHP and DBP exposures.

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1. Introduction

In 2008, the National Research Council published a report titled 'Phthalates and Cumulative Risk Assessment: the Task Ahead' (NRC, 2008). In this report, the panel concluded that phthalates met the conditions necessary to warrant a cumulative risk approach—the general population is exposed to multiple different phthalates, and these phthalates may contribute to common adverse outcomes. Although the report focused on effects related to the 'phthalate syndrome' of disrupted male reproductive development, there is evidence from both animal and human studies that phthalates impact a wide variety of health endpoints (see recent reviews including: (Jurewicz and Hanke, 2011; Lyche et al., 2009; Martino-Andrade and Chahoud, 2010; Meeker et al., 2009; Pak et al., 2011)).

One approach to estimating cumulative risk for non-cancer outcomes, from multiple exposures to toxicologically similar chemicals, is the hazard index (HI) approach which assumes dose addition (EPA, 2003, 2007; Teuschler and Hertzberg, 1995). As outlined in the NRC report, the HI provides a straightforward method to relate intake of a group of substances to their reference values (RfVs) (NRC, 2008) and this approach has been previously demonstrated in the literature (Kortenkamp and Faust, 2010; Soeborg et al., 2012). Example RfVs for oral exposure include the US Environmental Protection Agency (EPA) Reference Dose, RfD, and the European Union (EU) Tolerable Daily Intake, TDI. For each exposure a hazard quotient (HQ) is calculated as the ratio of the estimated exposure level to the RfV for that chemical. The chemical-specific HQs are then summed to estimate the overall summary HI. Guidance documents for conducting cumulative risk assessments emphasize that a final step is the interpretation of results (EPA, 2003, 2007). In this paper, we focus on the generation of the

Abbreviations: AGD, anogenital distance; BBP, butyl benzyl phthalate; CE, creatinine excretion; DBP, di-n-butyl phthalate; DEHP, di(2-ethylhexyl) phthalate; DEP, diethyl phthalate; DI, daily intake; DNEL, Derived No Effect Level; DiBP, diisobutyl phthalate; DiNP, diisononyl phthalate; EPA, Environmental Protection Agency; EU, European Union; FUE, fraction excreted in urine; HI, hazard index; HQ, hazard quotient; LOAEL, lowest observed adverse effect level; MBP, mono-n-butyl phthalate; MBZP, mono-benzyl phthalate; MCOP, mono-(carboxyoctyl) phthalate; MEP, monoethyl phthalate; MECPP, mono-(2-ethyl-5-carboxypentyl) phthalate; MEHHP, mono-(2-ethyl-5-hydroxyhexyl) phthalate; MEHP, mono-(2-ethylhexyl) phthalate; MEOHP, mono-(2-ethyl-5-oxohexyl) phthalate; MEP, monoethyl phthalate; MIBP, monoisobutyl phthalate; MINP, monoisononyl phthalate; MW, molecular weight; NHANES, National Health and Nutritional Evaluation Survey; NCHS, National Center for Health Statistics; NOAEL, no observed adverse effect level; POD, point of departure; RfD, Reference Dose; RfV, reference value; TDI, Tolerable Daily Intake; US, United States.

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quantities that go into the cumulative risk assessment – phthalate-specific intake estimates, HQs, and HIs. Regarding the interpretation, both the HQ and HI have practical interpretations and uses within a public health and regulatory context. These interpretations and uses derive from the careful wording of the definitions of these RfVs. For example, the EU defines the TDI as follows: "*A TDI is an estimate of the amount of a substance in air, food or drinking water that can be taken in daily over a lifetime without appreciable health risk. TDIs are calculated on the basis of laboratory toxicity data to which uncertainty factors are applied*" (EU, 2014). Therefore, if an individual's daily exposure is less than the TDI (i.e., the HQ is less than one), it is often concluded that this level of exposure is not likely to cause harmful effects during a lifetime. Similarly, if the HQ is found to be less than one for all individuals within a defined population, one might conclude that this exposure would not be of concern over their lifetimes. However, if the exposure is greater than the TDI (i.e., the HQ exceeds one), this does not imply that a health effect will occur. Several additional considerations include, among other things, whether the exposure is ongoing; whether the health effect used in developing the TDI is relevant for the exposed individual; and what uncertainty factors were used in developing the TDI. Similarly, an HI at or above one for a group of contaminants may indicate the need for further investigation, such as to take into account the degree of toxicological similarity, the appropriateness of dose additivity, and other issues.

In order to estimate the HQ and HI, it is necessary to know the exposure level in the population of interest. There are two general approaches used to estimate phthalate exposure. The 'forward' approach combines information on the concentration of phthalates in exposure media (including food, water, air, etc.) with exposure media contact rates (see for example, (Clark et al., 2011; Wormuth et al., 2006)). This approach requires that both the exposure sources and the concentrations of phthalates for each source are known. This information is often not available or is not of sufficient quality. Concentrations may be widely varied according to factors such as geographic region, distribution and use of products containing phthalates, and other issues. Further, laboratory equipment and reagents may themselves contain phthalates, which could lead to sample contamination (Guo and Kannan, 2012). This may bring into question the validity of exposure media measurements of phthalates, particularly phthalates in food. The 'backward' approach uses human biomonitoring data in combination with human metabolism information to extrapolate backward to the 'dose' which would have resulted in the observed biomarker level. For phthalates, the biomarkers used are generally phthalate metabolites present in urine. By measuring metabolites rather than parent compounds, this approach circumvents the contamination issue (Koch and Calafat, 2009). Additionally, the measurement of phthalate metabolites in urine provides an integrated measure of phthalate exposure from all sources (known and unknown), and incorporates individual variability in exposure profiles.

In the US, the majority of general population exposure comes from six specific phthalates: diethyl phthalate, DEP; di(2-ethylhexyl) phthalate, DEHP; di-n-butyl phthalate, DBP; diisobutyl phthalate, DiBP; diisononyl phthalate, DiNP; and butyl benzyl phthalate, BBP. In the nationally representative National Health and Evaluation Survey (NHANES), the metabolites of these phthalates show the highest levels among the phthalate metabolites measured (CDC, 2013b), and a recent study of estimated dietary exposure also identified these six as having the highest potential for exposure (Schecter et al., 2013). In this paper, we estimate daily intakes for five of these phthalates for the US population using the 'backward' approach applied to measurements in the NHANES, then estimate individual and population HQs and HIs for these phthalates; DEP is not included because in toxicology studies, it has not been shown to cause effects within the phthalate syndrome, a constellation of male developmental reproductive effects (NRC, 2008). We also look at the results for different population groups, including all adults (>18 years), women of approximate reproductive age (18–39 years), and children (6–11 years). We used two sources for health RfVs, EU TDIs and Denmark EPA Derived No Effect Levels (DNELs). Our rationale for selecting these two sources includes these considerations: (1) the RfVs were derived within the past 10 years, (2) the RfVs were developed based on effects within the "phthalate syndrome", (3) although the RfVs from these two sources are not derived by exactly the same methodology, they were consistently derived by each governing body. We selected two sources of RfVs for comparison to highlight potential differences in approach to deriving RfVs and subsequent variability in the resulting HQ/HI estimates. The available US EPA's RfDS were not used for this analysis because they were not all developed based on the phthalate syndrome. For example, the EPA RfD for DEHP was developed based on increased relative liver weight in guinea pigs (Carpenter et al., 1953; EPA, 2013). Finally, we explore the impact of selecting an alternative RfV for one of the phthalates, DEHP, on estimated hazard. This phthalate was selected for the impact analysis because it was found to drive the cumulative exposures and risk, as discussed below.

2. Materials and methods

Total exposure to phthalates has been studied primarily with the measurement of phthalate metabolites in urine. The phthalate metabolites, rather than the parent compound, are measured in urine because the parent compound is metabolized very quickly, before being excreted, and also due to issues of contamination from phthalates present in plastic laboratory equipment. One complexity is that these metabolites are not entirely specific—that is, more than one parent compound may degrade to a common metabolite. However, this is more the exception than the rule, and for each phthalate in our assessment, specific metabolites are identified which correspond to only the single parent. This section describes the method used to infer daily intakes from spot samples of phthalate metabolites, and applies that method to the NHANES database. The NHANES is a nationally representative complex sample survey of the civilian, non-institutionalized US population, and is maintained by the National Center for Health Statistics (CDC, 2013a). The current NHANES is a continuous cycle of surveys conducted every 2 years. Starting with the 1999–2000 survey, phthalates have been measured via spot urine sample in a one-third random sample of NHANES participants aged 6 years and older. Implications of the use of these data are discussed in Section 4. For this analysis, the cycles from 2005–2006 and 2007–2008 were used—earlier surveys were not included because measurements of the metabolites of DiNP were not available until 2005. In order to generate nationally representative estimates of daily phthalate intake, statistical survey procedures are used to account for sampling-associated variability, using the sampling strata and primary sampling unit information, and sampling weights provided by the National Center for Health Statistics (NCHS). All analyses were performed using the SAS statistical software package.

The primary method to back calculate estimated phthalate intake corresponding to a given measurement of phthalate metabolite in urine is known as the 'creatinine correction' approach (David, 2000; Kohn et al., 2000). The key assumption behind this approach is that phthalate intakes and eliminations are at steady state, such that the daily intake is equal to the daily elimination (with proper correction for elimination of metabolite versus intake of parent compound). Much data exist to support this assumption,

including data on phthalates in exposure media, near 100% occurrence frequency of phthalate metabolites in urine, and evidence that urine is the primary elimination mechanism in the body. These metabolites have elimination half-lives on the order of hours (e.g., (Koch et al., 2005, 2012)), which means that an exposure to a phthalate is followed by excretion of phthalate-specific metabolites in a matter of hours after that exposure.

For phthalates, the use of the creatinine correction approach requires knowledge of the relationship between the parent phthalate and subsequent metabolite excretion in urine. The fraction of parent which is metabolite excreted in urine (FUE) over a 24-h period, on a molar basis, has been estimated from human and animal studies; values are presented in Table 1 along with the molecular weights of the parent compound, MW_{parent}, and the metabolite, $MW_{metabolite}$.

The FUE values for metabolites of BBP are taken from a 24-person (all adults) controlled dosing study (Anderson et al., 2001) and the values for DEHP and DiNP metabolites from a 20-person (all adults) controlled dosing study (Anderson et al., 2011). The FUE values for metabolites of DiBP and DBP (Koch et al., 2012) are taken from controlled dosing studies in a single volunteer. For each of these studies, volunteers were given a single oral dose, and FUEs are calculated from urine collections in the subsequent 24 h. For each phthalate with the exception of DEHP, there is a single metabolite considered in the estimation of daily intakes. Human metabolism studies have shown that the simple monoester metabolites of the short-chain phthalates (e.g., DnBP → MnBP, DiBP → MiBP, and BBzP → MBzP), are the major metabolites—in the range of 70–85% of the oral dose is excreted as these metabolites. Hence, the 24-h fractions excreted in urine of these phthalates are high, as seen in Table 1. In contrast, the simple monoester metabolite accounts for <10% of the excretion for long-chain phthalates. Included in this category are DEHP and DiNP. For this reason, the secondary, oxidized metabolites for these phthalates are now being measured in exposure studies. For DiNP, two metabolites are measured, but one of them, the simple monoester MiNP, was only quantified in about 10% of the samples in the database used in this evaluation. For this reason, the secondary metabolite associated with DiNP, MCOP, is used for purposes of extrapolation. For DEHP, multiple metabolites, up to four, are typically measured in exposure studies. We used all four metabolites in the extrapolations done in this analysis.

Given this information and set of assumptions, along with the spot urine creatinine concentration (CrConc, expressed in mg/dL urine) and phthalate metabolite concentration in urine (PhMet, expressed in ng/mL urine), daily intake in μg/kg-day is estimated using this formulation of the creatinine correction equation (David, 2000; Wittassek et al., 2007, 2011):

$$DI_{phthalate} = ([PhMet_{cr-adj} * CE]/[Fue * 1000\ mg/g]) * MW_{parent}/MW_{metabolite} \quad (1)$$

where $DI_{phthalate}$ = the daily phthalate intake, μg/kg-day; $PhMet_{cr-adj}$ = the phthalate metabolite expressed on a creatinine basis, μg/g creatinine; CE = the daily creatinine excretion normalized to body weight, mg/kg-day; FUE = the fraction urinary excretion, expressed as molar fraction of the ingested phthalate excreted in urine over 24 h following exposure; 1000 = units conversion factor, mg/g; $MW_{parent,metabolite}$ = the molecular weights of the parent phthalate and metabolite, mg/mole.

Application of this approach for an individual person requires assignment of CE and $PhMet_{cr-adj}$. The 24-h creatinine excretion (CE) is based on demographic information (gender, age, race, height, and weight), using equations from Mage et al. (2008). Urine metabolite concentrations may already be provided in the proper units of μg/g creatinine, or may be calculated by dividing a volume based concentration (ng/mL, for example) by the concentration of creatinine in the urine sample (mg/dL, for example) with proper conversion.

For some phthalates such as DEHP, multiple metabolite concentrations are available. In this case, one can estimate the phthalate intake from each individual metabolite, or one can take advantage of all the information present by summing the concentrations of the measured metabolites. For this study, $PhMET_{cr-adj}$ for DEHP was calculated as the sum of the four DEHP metabolites divided by the creatinine concentration, FUE was the sum of the individual metabolite FUE, and the $MW_{metabolite}$ was calculated as the FUE-weighted average molecular weight of the metabolites used in the calculation.

Table 1
Molecular weights and urinary excretion fractions for phthalate metabolites.

Parent phthalate Compound	Phthalate monoester metabolite	Molecular weight of diester parent compound, g/mole (MW_{parent})	Molecular weight of metabolite, g/mole ($MW_{metabolite}$)	Urinary excretion fraction (FUE, 24-h), expressed as percent* (%)
Di-2-ethylhexyl phthalate (DEHP)	Mono-(2-ethylhexyl) phthalate (MEHP)	390.56	278.34	6.2
	Mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP)	390.56	294.35	14.9
	Mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP)	390.56	292.33	10.9
	Mono (2-ethyl-5-carboxypentyl) phthalate (MECPP)	390.56	308.33	13.2
Di-n-butyl phthalate (DnBP or DBP)	Mono-n-butyl phthalate (MBP)	278.34	222.24	84
Butylbenzyl phthalate (BBP)	Mono-benzyl phthalate (MBzP)	312.36	256.25	73
Di-isononyl phthalate (DiNP)	Mono-(carboxyoctyl) phthalate (MCOP)	418.61	322.36	9.9
Di-iso-butyl phthalate (DiBP)	Mono-iso-butyl phthalate (MiBP)	278.34	222.24	70.3

* The FUEs are taken from the following studies: DEHP (Anderson et al., 2011); DBP (Koch et al., 2012); BBP (Anderson et al., 2001); DiNP (Anderson et al., 2011); DiBP (Koch et al., 2012).

2.1. Estimation of the hazard quotients and the hazard index

The RfVs used to construct the HQ and HI are listed in Table 2. There are two different sets of RfVs—the EU TDIs and Denmark EPA DNELs. As described above, we selected these sets of RfVs because they are relatively recently derived, and because with one exception (the TDI for DiNP), they comprise RfVs for the different phthalates that are based on endpoints in the reproductive/developmental domain. For each set of RfVs, the HQ for an individual phthalate is calculated as the ratio of the estimated daily intake for each person in the NHANES, divided by the RfV for that phthalate. The HI is calculated by summing these individual-level HQs for each phthalate. We calculated HQs and HIs for the whole NHANES population (ages 6 years and above), as well as for different age groups and for men and women of approximate reproductive age (18–39 years). Note that because there is no TDI available for DiBP, and because the TDI for DiNP is based on liver effects, the TDI-based HI is the sum of the HQs for the other three phthalates (DEHP, DBP, BBP). This is not to imply that other types of health effects (aside from those in the phthalate syndrome) are not important, rather that reference values have not been consistently developed for other endpoints.

DEHP is one of the most important phthalates to consider in evaluating intake and potential risk of adverse health outcomes, since previous work has shown that exposure to DEHP is generally higher compared to other phthalates (CDC, 2013b), and along with DBP, has the lowest reference value among the phthalates studied here: DEHP and DBP have EU TDIs at 50 and 10 μg/kg-day, respectively, compared to 500 and 150 μg/kg-day for BBP and DiNP, respectively. Regarding exposure, the CDC's National Exposure Report shows that in 2009–2010, median urinary levels of four DEHP metabolites ranged from 1.5 to 20.4 ng/mL, compared with medians below the limit of detection, to 15.9 ng/mL for other metabolites surveyed (CDC, 2013b). The only metabolite with higher median levels was monoethyl phthalate (primary metabolite of diethyl phthalate, DEP), where the median was 54.9 ng/mL. Finally, numerous studies on the health effects of DEHP have been published in recent years, and even since the Denmark EPA values were published in 2009. Thus, it appears that DEHP and DBP are the major contributors to the HI in this analysis. We chose to investigate the impact of an alternate value for DEHP as an example, recognizing that a similar exercise could be performed for DBP (or multiple phthalates). We performed an updated literature search to identify new (i.e., published between January 2009 and April 2013) studies which could potentially support derivation of an oral reference value for DEHP and that postdated the EU and Denmark RfV decision documents. The literature search and study evaluation were not intended to be an exhaustive and rigorous systematic review of all literature on DEHP, but were undertaken with the intent of identifying a study or set of studies which were judged to be methodologically sound and relevant to human health, and which reported sufficient quantitative data to derive a reference value. This is not to say that our search and evaluation strategy would capture all such studies, but findings from this exercise may provide a sense for the potential variation in the HI (and DEHP HQ). Studies identified through the literature search were evaluated for methodological robustness, strength of evidence for observed responses to treatment (within each study as well as within the overall database), adversity of outcomes, and dose response characteristics. Although not strictly adhering to any formal risk assessment protocol, this approach to study evaluation and selection is generally consistent with established guidelines such as those developed by the U.S. EPA for evaluating developmental toxicity (EPA, 1991b), reproductive toxicity (EPA, 1996), and neurotoxicity (EPA, 1998).

We identified three DEHP studies in rats that reported effects on male reproductive system development at parental doses of <15 mg/kg/day DEHP (Blystone et al., 2010; Christiansen et al., 2010; Gray et al., 2009). Blystone et al. (2010) found a dose-related increase in the combined incidence of reproductive tract malformations observed at postmortem assessment of adult F1 and F2 male rats from a multigenerational reproductive assessment by continuous breeding (RACB) (NTP, 2005). Similarly, Gray et al. (2009) reported an increased incidence of reproductive tract malformations and related abnormalities in F1 adult males that had been administered DEHP during *in utero* and early postnatal development. The study by Christiansen et al. (2010), which administered DEHP to dams during gestation and lactation and examined (without knowledge of treatment group) the genitalia of male offspring at postnatal day 16, revealed treatment-related increases in the litter incidence of external genital dysgenesis. Derivations of candidate oral RfV's from these three studies are shown in Table 3. Following standard EPA practice (EPA, 2002), the LOAELs of 3 (Christiansen et al., 2010) or 11 (Gray et al., 2009) mg/kg-day were divided by a composite uncertainty factor (UF) of 1000, comprised of an interspecies UF of 10, intraspecies UF of 10, subchronic

Table 2
Reference values (μg/kg-day) used in derivation of hazard quotients and hazard indices.

Phthalate, source	EU: (EFSA, 2005a,b,c,d) Tolerable Daily Intake	Denmark EPA: (Tønning et al., 2009)* Derived No Effect Level
DEHP	TDI = 50 μg/kg-day (Testis-related parameters: ↓ testicular weight, small or aplastic testes, seminiferous tubular atrophy, infertility) (EFSA, 2005c) NOAEL = 5 mg/kg-day (5000 μg/kg-day) Total UF = 100	DNEL = 50 μg/kg-day (effects on gametes and ↓ testicular weight) (Wolfe and Leyton, 2003) NOAEL = 5 mg/kg-day (5000 μg/kg-day) AF1 = 4, AF2 = 2.5, AF3 = 10, AF4 = 1 Total UF = 100
DBP	TDI = 10 μg/kg-day (↓ Number of spermatocytes) (EFSA, 2005b) LOAEL = 2 mg/kg-day (2000 μg/kg-day) Total UF = 200	DNEL = 6.7 μg/kg-day (effects on gamete development and development of mammary tissue) (Lee et al., 2004) LOAEL = 2 mg/kg-day (2000 μg/kg-day) AF1 = 4, AF2 = 2.5, AF3 = 10, AF4 = 3 Total UF = 300
BBP	TDI = 500 μg/kg-day (↓ Anogenital distance) (EFSA, 2005d) NOAEL = 50 mg/kg-day (50,000 μg/kg-day) Total UF = 100	DNEL = 500 μg/kg-day (↓ AGD) (Tyl et al., 2004) NOAEL = 50 mg/kg-day (50,000 μg/kg-day) AF1 = 4, AF2 = 2.5, AF3 = 10, AF4 = 1 Total UF = 100
DiNP	TDI = 150 μg/kg-day (↑ Incidence of spongiosis hepatis, accompanied by ↑ serum levels of liver enzymes and absolute and relative liver and kidney weights in both sexes) (EFSA, 2005a) NOAEL = 15 mg/kg-day (15,000 μg/kg-day) Total UF = 100	DNEL = 1600 μg/kg-day (↓ testicular weight) (Arnstech, 1994) NOAEL = 276 mg/kg-day (276,000 μg/kg-day) AF1 = 7, AF2 = 2.5, AF3 = 10, AF4 = 1 Total UF = 175
DiBP	–	DNEL = 1250 μg/kg-day (↓ AGD and ↑ nipple retention) (Saillenfait et al., 2008) NOAEL = 125 mg/kg-day (125,000 μg/kg-day) AF1 = 4, AF2 = 2.5, AF3 = 10, AF4 = 1 Total UF = 100

* Note: Focus on animal studies of ED effects. Assessment factors (AFs) are (1) interspecies (4 for rats, 7 for mice), (2) interspecies (2.5 for 'remaining interspecies differences'), (3) intraspecies (10), (4) dose response (3 for LOAEL to NOAEL).

Table 3
Derivation of alternative reference values for DEHP.

Citation	Endpoint	POD (μg/kg/day)	UF_A	UF_H	UF_L	UF_S	UF_D	Total UF	RfV
Christiansen et al. (2010)	Increased litter incidence of mild external genital dysgenesis in F1 male pups on PND 16	LOAEL = 3000	10	10	10	1	1	1000	3
Gray et al. (2009)	Increased incidence of F1 male pups with any phthalate syndrome finding on PND 64	LOAEL = 11,000	10	10	10	1	1	1000	10
Blystone et al. (2010)	Increased litter incidence of reproductive tract malformations in F1 and F2 adult male offspring	NOAEL = 4900[a]	10	10	1	1	1	100	50

Key to Uncertainty Factors (UF): UF_A = interspecies; UF_H = intraspecies; UF_L = LOAEL to NOAEL; UF_S = subchronic to chronic; UF_D = database (EPA, 2002).
[a] Dose estimate is based upon F1 parental test substance intake.

to chronic UF of 1 (due to use of a developmental effect), LOAEL to NOAEL UF of 10 (since a NOAEL was not identified for the critical effect), and database UF of 1 (i.e., based on an assumption that there are no substantial data gaps in the oral toxicology database for DEHP). The NOAEL of 4.9 mg/kg-day (Blystone et al., 2010) used the same adjustments, with the exception that the LOAEL to NOAEL UF was 1, for a composite UF of 100. Since the Christiansen et al. (2010) study identified a biomarker of developmental disruption in the male reproductive tract that was not examined using the same specific evaluation protocol (nor evaluated at the same life stage) by Blystone et al. (2010) and Gray et al. (2009) studies, we focused on this study to select an 'alternative' RfV for DEHP. Also, it resulted in the lowest RfV (3 μg/kg-day) of the three studies, so its use demonstrates the impact of a substantially lower RfV for DEHP.

3. Results

The intake estimates using NHANES data are shown in Table 4. This table provides the nationally representative estimates of intake using the survey weights; estimates based on the unweighted survey data were similar (data not shown). DEHP had the highest intakes, with medians in the range of about 5–15 μg/kg-day, and 95th percentiles in the 30–50 μg/kg-day range. Estimated intake of each phthalate on a body weight basis was higher for children (6–11 years) compared to the total study group (⩾6 years); this was true both for median and extreme (95th percentile) intake estimates. Men and women of approximate reproductive ages (18–39 yrs) had similar estimated intakes for all phthalates. Each of the phthalates showed considerable variation in intake, as indicated by the magnitude of the standard deviations, the difference between the means and medians, and the width of the interquartile range (IQR). This variability is due to the skewed nature of the distribution of urinary metabolite concentrations and subsequent estimated intakes; a small number of very high estimated intakes (based on very high urine concentrations) was observed in some cases. For example, the maximum estimated intake for DBP was 3265 μg/kg-day, nearly three orders of magnitude above the 95th or 99th percentiles.

Table 5a shows the median, interquartile range (25th and 75th percentiles), and the 95th percentile of the HQ for each of the options for HQ calculation plus the analysis in which the DEHP TDI and DNEL of 50 μg/kg-day is replaced by the alternate value of 3 μg/kg-day, and for each of the NHANES population groups.

The HIs calculated for each RfV approach (with the original and with the alternate value for DEHP) are shown in Table 5b; as noted in the Methods, the HI calculations do not include DiNP since the critical effect is not in the reproductive/developmental domain. Note that the descriptive statistics are based on estimates calculated for each individual in the NHANES and therefore do not correspond to a simple sum of the phthalate-specific HQs

Table 4
Estimated daily intake of phthalates (ug/kg-day), NHANES 2005–2008, accounting for survey weighting.[*]

Phthalate (metabolites)	Population	Mean (±SD)	Median	25th–75th Percentile	95th Percentile
BBP (MBzP)	All ages ⩾6 yrs	0.60 (8.01)	0.2	0.1–0.5	1.0
	Ages 6–11 yrs	1.12 (1.99)	0.7	0.3–1.3	3.4
	Women, ages 18–39 yrs	0.48 (0.74)	0.3	0.1–0.5	1.5
	Men, ages 18–39 yrs	1.38 (19.56)	0.3	0.1–0.5	1.3
DBP (MBP)	All ages ⩾6 yrs	1.34 (37.48)	0.5	0.3–0.9	2.1
	Ages 6–11 yrs	6.0 (124.54)	0.9	0.6–1.4	3.5
	Women, ages 18–39 yrs	0.89 (1.02)	0.6	0.4–1.0	2.2
	Men, ages 18–39 yrs	1.20 (10.46)	0.5	0.3–0.9	1.6
DiBP (MiBP)	All ages ⩾6 yrs	0.46 (7.51)	0.2	0.1–0.4	0.8
	Ages 6–11 yrs	0.55 (1.03)	0.4	0.2–0.6	1.49
	Women, ages 18–39 yrs	0.36 (0.51)	0.2	0.1–0.4	0.9
	Men, ages 18–39 yrs	1.21 (18.38)	0.2	0.1–0.4	0.8
DEHP (MEHP, MEOHP, MEHHP, MECPP)	All ages ⩾6 yrs	9.67 (24.79)	3.5	2.0–7.6	34.5
	Ages 6–11 yrs	11.16 (19.79)	6.0	3.7–10.7	40.9
	Women, ages 18–39 yrs	11.95 (33.40)	4.2	2.5–8.9	34.5
	Men, ages 18–39 yrs	11.42 (24.61)	3.9	2.2–9.3	48.7
DiNP (MCOP)	All ages ⩾6 yrs	3.25 (10.51)	1.3	0.7–2.7	11.7
	Ages 6–11 yrs	4.76 (24.22)	2.5	1.5–4.4	13.0
	Women, ages 18–39 yrs	3.69 (8.90)	1.3	0.7–2.8	13.7
	Men, ages 18–39 yrs	3.66 (8.80)	1.4	0.8–3.1	13.9

[*] Sample size for all ages ⩾6 yrs is n = 5109 unweighted, n = 262,575,752 (SD = 11,465,204) weighted. Sample size for ages 6–11 yrs is n = 742 unweighted, n = 23,350,727 (SD = 1,413,455) weighted. Sample size for women ages 18–39 yrs is n = 754 unweighted, n = 42,738,565 (SD = 2,511,187) weighted. Sample size for men ages 18–39 yrs is n = 676 unweighted, n = 43,514,493 (SD = 2,620,703) weighted.

Table 5a
Hazard Quotients* calculated for each set of reference values.

Phthalate	Population*	EU TDI		Denmark EPA DNEL	
		Median (25th–75th percentile)	95th percentile	Median (25th–75th percentile)	95th percentile
BBzP	All ages ⩾6 yrs	0.0005 (0.0002–0.001)	0.003	0.0005 (0.0002–0.001)	0.003
	Ages 6–11 yrs	0.001 (0.0007–0.003)	0.007	0.001 (0.0007–0.003)	0.007
	Women, ages 18–39 yrs	0.0006 (0.0003–0.001)	0.003	0.0006 (0.0003–0.001)	0.003
	Men, ages 18–39 yrs	0.0006 (0.0003–0.001)	0.002	0.0006 (0.0003–0.001)	0.002
DBP	All ages ⩾6 yrs	0.05 (0.03–0.09)	0.21	0.08 (0.05–0.13)	0.32
	Ages 6–11 yrs	0.09 (0.06–0.14)	0.35	0.14 (0.08–0.22)	0.52
	Women, ages 18–39 yrs	0.06 (0.04–0.10)	0.22	0.09 (0.06–0.15)	0.33
	Men, ages 18–39 yrs	0.05 (0.03–0.09)	0.16	0.08 (0.05–0.13)	0.23
DiBP	All ages ⩾6 yrs	–	–	0.0002 (0.0001–0.0003)	0.0007
	Ages 6–11 yrs	–	–	0.0003 (0.0002–0.0005)	0.001
	Women, ages 18–39 yrs	–	–	0.0002 (0.0001–0.0003)	0.0007
	Men, ages 18–39 yrs	–	–	0.0002 (0.0001–0.0003)	0.0006
DEHP	All ages ⩾ 6 yrs	0.07 (0.04–0.15)	0.69	0.07 (0.04–0.15)	0.69
	Ages 6–11 yrs	0.12 (0.07–0.22)	0.81	0.12 (0.07–0.21)	0.81
	Women, ages 18–39 yrs	0.08 (0.05–0.18)	0.69	0.08 (0.05–0.18)	0.69
	Men, ages 18–39 yrs	0.08 (0.04–0.19)	0.94	0.08 (0.04–0.19)	0.97
DiNP	All ages ⩾6 yrs	0.01 (0.005–0.02)	0.08	0.001 (0.0004–0.002)	0.007
	Ages 6–11 yrs	0.02 (0.01–0.03)	0.09	0.002 (0.001–0.003)	0.008
	Women, ages 18–39 yrs	0.01 (0.005–0.02)	0.09	0.001 (0.0005–0.002)	0.009
	Men, ages 18–39 yrs	0.01 (0.005–0.02)	0.09	0.001 (0.0005–0.002)	0.009
DEHP. Alternate value†	All ages ⩾6 yrs	1.18 (0.65–2.52)	11.50	1.18 (0.65–2.52)	11.50
	Ages 6–11 yrs	1.99 (1.23–3.53)	13.58	1.99 (1.23–3.53)	13.58
	Women, ages 18–39 yrs	1.41 (0.82–2.95)	11.48	1.41 (0.82–2.95)	11.48
	Men, ages 18–39 yrs	1.31 (0.73–3.10)	15.69	1.31 (0.73–3.10)	15.69

* Estimates from the 2005–2008 National Health and Nutrition Examination Survey, accounting for survey weighting. Estimates are calculated for each individual in the NHANES, and therefore do not correspond to a simple ratio of median intake to RfD.
† Alternative RfV for DEHP developed based on Christiansen et al. (2010).

Table 5b
Hazard Indices* calculated for each set of reference values.

	EU TDI		EU TDI with alternate value for DEHP†		Denmark EPA DNEL		Denmark EPA DNEL with alternate value for DEHP	
	Median (25th–75th percentile)	95th percentile	Median (25th–75th percentile)	95th percentile	Median (25th–75th percentile)	95th percentile	Median (25th–75th percentile)	95th percentile
All ages ⩾6 yrs	0.14 (0.09–0.26)	0.87	1.28 (0.72–2.64)	11.71	0.18 (0.11–0.31)	0.96	1.32 (0.75–2.70)	11.76
Ages 6–11 yrs	0.24 (0.15–0.37)	1.06	2.05 (1.33–3.68)	14.29	0.30 (0.18–0.45)	1.15	2.15 (1.37–3.74)	14.36
Women, ages 18–39 yrs	0.17 (0.11–0.31)	0.86	1.54 (0.90–3.08)	11.68	0.22 (0.14–0.37)	1.06	1.57 (0.92–3.10)	11.76
Men, ages 18–39 yrs	0.15 (0.10–0.27)	1.10	1.37 (0.78–3.15)	16.55	0.20 (0.12–0.33)	1.14	1.42 (0.81–3.22)	16.58

* Estimates from the 2005–2008 National Health and Nutrition Examination Survey, accounting for survey weighting. Estimates are calculated for each individual in the NHANES, and therefore do not correspond to a simple sum of HQs shown in Table 4a.
† Alternative RfV for DEHP developed based on Christiansen et al. (2010).

shown above. For both the TDIs and the DNELs, the HQs were notably higher for DEHP and DBP (TDI: median 0.05–0.12, DNEL: medians 0.07–0.14) compared to BBzP and DiNP (TDI: median <0.02, 95th percentile <0.1, DNEL: medians and 95th percentiles <0.1).

DEHP was found to contribute the most to the HI for both RfV options (Supplementary Table 1). For the HI based on TDIs, the median contribution from DEHP was 57.4% (IQR: 41.7–75.0%), and DBP also had a substantial contribution at 41.8% (median; IQR: 24.6–57.4%). For DNELs, the contribution from DEHP and DBP were similar (medians of 46.8% and 51.1%, respectively).

Tables 5a and b show the impact of substituting the alternative value for the DEHP RfV of 3 μg/kg-day. As expected from this lower value, the HQ for DEHP increased, with median values between 1.2 and 2, and 95th percentiles between 11.5 and 15.7 (Table 5a). Similarly, the HI also increased, with median values exceeding 1 in all groups, and 95th percentiles between 11.7 and 16.5; the proportion of the HI contributed by DEHP was >90% in all groups (Table 5b).

4. Discussion

The HQ and HI approaches provide a simple, intuitive method to evaluate noncancer risk for a given level of exposure to a substance or a toxicologically similar group of substances, respectively. Calculating and interpreting the HQ and HI depend upon the method used to estimate level of exposure, as well as the choice of a reference value. We used data from the 2005 to 2008 NHANES to estimate intake of five different phthalates based on phthalate metabolite levels in urine spot samples; this information was combined with two sets of reference values to demonstrate the potential for cumulative risk from exposure to phthalate mixtures using the HQ/HI approach in a U.S. nationally representative dataset.

The 'creatinine-correction' approach is a commonly used method to estimate daily intake of phthalates and similar contaminants which have these characteristics – excretion is primarily through urine and complete within hours or days. However, there are uncertainties to consider when using the creatinine correction

approach. Phthalate metabolites have very short half-lives, on the order of ~5 to 12 h (Koch et al., 2005; Volkel et al., 2002, 2005). Thus urinary concentrations peak shortly after exposure (Kluwe, 1982; Koch et al., 2005) and urine sampled during this time of peak concentration could lead to artificially high estimates of daily intake. Conversely, measurements made after concentrations have peaked and declined could lead to artificially low intake estimates. The creatinine correction approach assumes a steady state condition exists; that is, a sampled concentration adequately represents a daily average concentration. This assumption was critically evaluated by Aylward et al. who obtained the raw data from a study in which 7 volunteers provided all urine samples over the course of 8 days (Aylward et al., 2012). These samples were measured for the DEHP metabolites MEHP and MEOHP, as well as creatinine. Aylward et al. compared the intakes derived from spot samples to intakes derived from a full day's volume or urine events (in conjunction with the metabolite concentrations and toxicokinetics). They found that intakes derived from spot samples ranged from between 1/5 as high to 3 times higher than intakes derived from a full day's excretion of metabolites. Said another way, daily intakes derived from spot samples could possibly range from 20% to 300% of the actual daily intake (assuming that a full day volume of urine events correctly interpreted is the best possible measure of daily intakes).

Although this variability may affect the accuracy of an estimated intake for a single individual, recent work has demonstrated that on the population level, a group of spot urine samples provides a reasonable approximation of concentrations that would have been observed in a population of full-day urine samples collected from the same population for phthalates and similar short-lived contaminants like bisphenol A (Christensen et al., 2012; Frederiksen et al., 2013). Addressing this issue from another angle, Teitelbaum et al. evaluated urinary concentrations of phthalate metabolites, phytoestrogens and phenols among 35 minority children over a 6-month period (Teitelbaum et al., 2008). They concluded that while concentrations did vary over time, a single spot sample was reliable in ranking subjects according to tertile of all the analytes studied, indicating that a single spot sample may recapitulate longer-term trends in exposure. Thus, while there may be variability in the tails of the distribution (i.e., the extreme highs and lows), the estimated central tendency for the population is likely to be rather stable. Similarly to previous studies of phthalate exposure (Koch et al., 2011; Lakind and Naiman, 2011; Marsee et al., 2006), we present findings for the 95th percentile of estimated phthalate intake, recognizing that there may be more variability in these values, because this information provides insight into the potential risk at the highest levels of exposure in a general population setting.

Another concern is the utility of creatinine as an adjustment factor across demographic groups, and in populations where creatinine excretion may be more widely variable, such as children (due to growth and muscle development) and pregnant women (due to changing body composition and volume expansion) (Barr et al., 2005; Braun et al., 2011). To address this concern, regression models have been developed to estimate daily creatinine excretion from spot sample concentrations, accounting for age, race, gender and body size (Mage et al., 2008). However, the fluctuations in creatinine excretion observed during pregnancy (Boeniger et al., 1993; Davison and Noble, 1981; Lohsiriwat and Imrittha, 2008) have not been fully addressed. On the other hand, there were very few pregnant women with phthalate measurements in the NHANES, and their inclusion is not likely to bias results greatly. Specifically with regards to children, the Mage equations used here (Mage et al., 2008) are age-group specific, and equations for children are based on creatinine excretion data collected in children described in Remer et al. (2002).

Finally, there are some general uncertainties with use of the creatinine approach for estimating daily intakes, particularly with the assignment of the FUE parameter. There is generally sparse information to determine values for this parameter for humans, and as noted above, the FUE values for metabolites of DiBP and DBP (Koch et al., 2012) are taken from controlled dosing studies in a single volunteer. There is an additional uncertainty associated with using the creatinine correction approach in children. The parameter, FUE, was assigned values based on controlled dosing experiments on adults. The metabolism of phthalates in children is likely different than in adults, but that difference will probably never be known due to ethical considerations of controlled dosing experiments with children. These uncertainties notwithstanding, the reliability of phthalate metabolite measurements as well as the national statistical representativeness of NHANES leads to the conclusion that back-calculated intakes using NHANES provide useful and valid estimates.

The uncertainties with development of RfV such as the DNEL, TDI, or RfD have been extensively addressed elsewhere (e.g., (De Rosa et al., 1999; EPA, 2002; OECD, 2011)). Generally, the starting point is to establish a point of departure (POD), which is then adjusted by a standard set of uncertainty factors (UFs). By example, for EPA these uncertainty factors may include: interspecies uncertainty factor, for extrapolation from animals to humans (UF_A); intraspecies uncertainty factor, for human variability (UF_H); subchronic to chronic uncertainty factor, for studies covering a less-than-lifetime period (UF_S); LOAEL to NOAEL uncertainty factor, for extrapolation from a 'lowest adverse effect level' to a 'no adverse effect level) (UF_L); database uncertainty factor, to account for deficiencies in the database of available studies (UF_D). Clearly, differences in RfVs may arise due to different practices by individuals or agencies in procedures or decisions applied in assigning these UFs, including: the application of 'standard' or 'default' UF values; the selection of the point(s) of departure; availability of information such as physiologically based pharmacokinetic models; the extent of the literature database; and so on. For this exercise, we elected to use default uncertainty factor values from the EPA guidelines. Derivation of RfVs incorporates consideration of uncertainty; indeed, the EPA definition of the RfD as "*an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime*" (EPA, 2002) is carefully worded to caution against over-interpretation.

Typically, a major area of uncertainty when using the HI is the assumption of dose-additivity. However, as compared to most chemical groups, this is less of a concern for phthalates because there are some toxicology studies which provide empirical evidence of the joint effect of multiple phthalates in relation to 'phthalate syndrome' effects (Hannas et al., 2011; Howdeshell et al., 2008; Rider et al., 2009). Each of these found that effects were best predicted by a dose-additive model, lending support to the use of the HI. However, it is not clear whether this additivity would hold for other health outcomes.

While the HQ and HI have the advantage of being very easily calculated and easily interpretable, there are also uncertainties associated with their use. First, they provide only a single number to characterize risk, due to the use of a single reference value in the denominator. Determining the distribution of risk within a population is a complex process that should consider variability within and between groups, and potential impact of factors such as age and gender. Also with regard to the reference values, the selection of endpoint has an obvious effect on the outcome of the exercise. The approach taken here was to use the reference values corresponding to a single health domain, effects associated with the 'phthalate syndrome.' However, one could, for example, choose

different endpoint domains for each exposure of interest based upon the quality of the database, or the magnitude of the reference value. Clearly the value of the RfVs can vary widely depending on such factors. As shown above, while DEHP exposure dominate the HI results, the results differ widely whether using the European TDI or Denmark EPA DNEL of 50 μg/kg-day, or our alternate value of 3 μg/kg-day. A similar sensitivity analysis to the one performed for DEHP, could be done for other phthalates, which may result in different RfVs and consequent impact on the estimated HQs and HIs.

RfVs and estimated exposure levels may be used (along with other information such as technological feasibility, economics, and characteristics of the exposure population) to inform policy and risk management decisions, and exceedances of RfVs, or HQ/HI values greater than 1.0, can be important. For example, EPA's Superfund Program (administered through the Office of Solid Waste and Emergency Response, OSWER), as part of their early scoping process, develops a 'Preliminary Remediation Goal' (PRG; (EPA, 1991a; EPA, 2004)), or a soil cleanup level, based on a modeled dose of a contaminant through exposure to soil and all other site-specific pathways (ground water ingestion, inhalation, etc.) using the RfD for that contaminant as a target exposure level, but acknowledging these may be modified significantly depending on information gathered about the site; i.e., a target goal that the HQ not exceed 1.0. Another example is the use of a additional tenfold "Children's Safety Factor "applied in certain circumstances to the RfD of a pesticide under the Food Quality Protection Act (EPA, 2006; FQPA, 1996), to consider increased sensitivity of children to exposure to that pesticide. In other words, the exposure calculated for a specific pesticide use scenario is of concern if it exceeds 1/10 the RfD (HQ of 0.1). This additional factor is now standard in dietary risk assessments, unless reliable data support a different factor.

The TDI developed by the European Food Safety Authority (EFSA) has been used in specific cases, including a recent case involving the phthalate DEHP. While the ban of toys containing phthalates by the EU (EU, 2005) has been well documented, the EU also investigated the exposure of children to DEHP in school supplies (EU, 2009). The Danish EPA analyzed phthalates and other contaminants (isophorone, butylated hydroxytoluene, cyclohexanone, phenol, and other substances) in school supplies such as school bags, play bags, pencil cases and erasers. They found DEHP was used in some school erasers as a plasticizer. Using data from a laboratory study on the migration of DEHP from school erasers and possible exposure routes of a child, they found, "Combining all these worst-case scenarios it results in an exposure of 4.1 mg/child or 0.2 mg/kg for a 6 years old child of 20 kg of weight. This exposure is 4-fold above the EFSA TDI of 0.05 mg/kg/day for DEHP and with a MOE (margin of exposure) of 25 to the NOAEL for DEHP of 4.8 mg/kg bw/day identified by CSTE (CSTE, 2004), derived with a major contribution from swallowed particles. Uptake of DEHP by licking only, even when using a conservative assessment, will not exceed the afore-mentioned highly conservative EFSA TDI for DEHP" (p. 8, (SCHER, 2008)). The EU concluded, "Since swallowing particles bitten off an eraser represents a short-time habit of children or even a one-time event, it is unlikely that such exposure leads to health consequences" (EU, 2009). The EU also found that other contaminants and pathways would not lead to exposures of concern, and no actions were taken.

With this as background, it is seen from analysis in this paper that the HIs for cumulative exposure to phthalates may exceed 1.0 at the 95th percentile using either the EU TDIs or the Danish EPA DNELs. Using an alternative RfV for DEHP of 3 μg/kg-day based on a recent toxicological study, a larger proportion of the population could have HIs exceeding 1.0, which could have implications for future risk management decisions.

5. Conclusions

We demonstrate a biomarker-based method to estimate daily intake of five different phthalates. Such exposure estimates can be used to generate HQ and HI estimates, in order to quantify potential risk from a single exposure, and cumulative risk across multiple chemical exposures. These measures provide an easily interpretable, straightforward approach to characterize risk, but depend on a number of assumptions (e.g., dose additivity) and are influenced by the reference values chosen. Uncertainties in the estimation of exposure (e.g., the assumption that a single spot sample characterizes average daily excretion) should also be considered. In this assessment, we showed that exposures to DEHP are a major contributor to cumulative exposure to the phthalates assessed, and thus to the overall hazard index. Based on use of the EU or Danish health reference values for DEHP, the HI at the median is about 0.3. If using an alternative reference value discussed above that was based on a recent toxicological study by Christiansen et al. (2010), the HI for median DEHP exposure could exceed 1.0.

Disclaimer

The views expressed in this manuscript are those of the authors and do not necessarily reflect the views or policies of the U.S. Environmental Protection Agency.

Conflict of interest

There is no conflict of interest to declare.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.yrtph.2014.04.019.

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